

What is Claimed is:

1. A method of producing a plurality of fused aggregates forming a desired three-dimensional structure, the method comprising:

depositing a layer of a matrix on a substrate;

embedding a plurality of cell aggregates, each comprising a plurality of cells, in the layer of the matrix, the aggregates being arranged in a predetermined pattern;

allowing at least one aggregate of said plurality of cell aggregates to fuse with at least one other aggregate of the plurality of cell aggregates to form the desired structure; and

separating the structure from the matrix.

2. The method of claim 1 wherein the layer of the matrix constitutes a first layer, the plurality of cell aggregates constitutes a first plurality of cell aggregates, and the predetermined pattern constitutes a first predetermined pattern, the method further comprising the steps of:

depositing a second layer of the matrix on the first layer; and

embedding a second plurality of cell aggregates in the second layer, the second plurality of cell aggregates comprising a plurality of cells, the second plurality of cell aggregates being arranged in a second predetermined pattern, and

allowing at least one cell aggregate in the first plurality of cell aggregates to fuse with at least one

cell aggregate in the second plurality of cell aggregates.

3. The method of claim 2 wherein the first and second predetermined patterns are substantially the same, and wherein the second plurality of cell aggregates is embedded in the second layer of the matrix in registration with the first plurality of cell aggregates.

4. The method of claim 2 wherein the desired structure is a tube, the first and second predetermined patterns are both circular in shape, and the second plurality of cell aggregates is embedded in the second layer of the matrix in registration with the first plurality of cell aggregates.

5. The method of claim 1 wherein the thickness of the layer of the matrix is about equal to the average diameter of the plurality of cell aggregates.

6. The method of claim 1 wherein the cell aggregates are substantially spherical.

7. The method of claim 1 wherein the cell aggregates are substantially uniform in size.

8. The method of claim 1 wherein the cell aggregates have an average size between about 100 and about 600 microns.

9. The method of claim 8 wherein no more than about 10% percent of the cell aggregates deviate from said average size by more than 5%.

10. The method of claim 8 wherein the cell aggregates have an average size between about 100 and about 500 microns.

11. The method of claim 1 wherein the cell aggregates consist essentially of cells of a single type.

12. The method of claim 1 wherein at least one of the cell aggregates comprises a plurality of cells of a first type and a plurality of cells of a second type that is different from the first type.

13. The method of claim 12 wherein said at least one cell aggregate comprises a mixture of said cells of the first type and said cells of the second type and the method further comprises the step of allowing at least some of the cells of the first type to segregate from at least some of the cells of the second type.

14. The method of claim 13 wherein the cells of the first type are epithelial cells and the cells of the second type are connective tissue-forming cells.

15. The method of claim 1 wherein the predetermined pattern comprises a ring.

16. The method of claim 1 wherein the matrix comprises a gel.

17. The method of claim 1 wherein said plurality of cell aggregates includes at least one cell aggregate consisting essentially of cells of a first type and at least one other cell aggregate consisting essentially of cells of a second type different from the first type.

18. A computerized method of producing a plurality of fused aggregates forming a desired three-dimensional structure, the method comprising:

selecting an embedding pattern by which a plurality of cell aggregates are to be embedded in a matrix, the plurality of cell aggregates each comprising a plurality of cells;

establishing a group of candidate matrices to be evaluated for use as the matrix based at least in part on the compatibility of the candidate matrices with the cell aggregates;

executing a computer simulation based on parameters comprising an interaction force between two of the cells of each of the plurality of cells, an interaction force between one of the cells and each of the candidate matrices, and an interaction force between two volume elements of each of the candidate matrices to predict structural evolutions of the pluralities of cells that are likely to occur if said plurality of cell aggregates are embedded in each of the candidate matrices in accordance with the embedding pattern; and

selecting one of the candidate matrices that is predicted to result in the cell aggregates evolving into the desired three-dimensional structure for use in producing fused aggregates forming the desired structure.

19. The method of claim 18 further comprising:
forming the matrix by depositing the selected candidate matrix on a substrate;
embedding said plurality of cell aggregates in the matrix in accordance with the embedding pattern;
allowing at least one aggregate of said plurality of cell aggregates to fuse with at least one other cell aggregate of said plurality of cell aggregates to form the desired structure; and
separating the structure from the matrix.

20. The method of claim 18 wherein said plurality of cells comprises a plurality of cells of a first type and a plurality of cells of a second type, wherein said interaction force between two of the cells comprises an interaction force between two cells of the first cell type, said interaction forces between one of the cells and each of the candidate matrices comprises interaction forces between a cell of the first type and the candidate matrices, and wherein said computer simulation is based on parameters further comprising a strength of an interaction force between a cell of the first type and a cell of the second type, a strength of an interaction force between two cells of the second type, and a strength of an interaction force between a cell of the second type and each of the candidate matrices.

21. The method of claim 20 wherein at least some of the candidate matrices are compound matrices comprising a combination of at least one volume of a first substance having a first set of characteristics and at least one volume of a second substance having a second set of characteristics, and wherein for each compound candidate matrix said interaction force between a cell of the first type and the candidate matrix is a strength of an interaction force between a cell of the first type and the first substance and said strength of an interaction force between a cell of the second type and the candidate matrix comprises an interaction force between a cell of the second type and the second substance, the embedding pattern being configured so that cells of the first type are substantially separated from said volume of the second substance by cells of the second type and cells of the second type are substantially separated from said volume of the first substance by cells of the first type.

22. The method of claim 18 wherein the step of executing a computer simulation comprises:

(i) creating a numerical model of a structure of said pluralities of cells and a first candidate matrix of the candidate matrices that is representative of an initial structure that said pluralities of cells and the first candidate matrix would have if said plurality of cell aggregates are embedded in the first candidate matrix in accordance with the embedding pattern;

(ii) estimating an initial total interaction energy of said pluralities of cells and the first candidate

matrix if said plurality of cell aggregates are embedded in the first candidate matrix in accordance with the embedding pattern;

(iii) simulating with a computer random movement of one of said pluralities of cells with respect to the remainder of said pluralities of cells and estimating a change in the total interaction energy caused by said movement;

(iv) updating the model to incorporate said movement if it lowers the total interaction energy; and

(v) repeating steps (iii) and (iv) until it is known whether the model evolves into a long-lived structure.

23. A computerized method of producing a plurality of fused aggregates forming a desired three-dimensional structure comprising:

identifying a matrix to be used to temporarily support a plurality of cell aggregates embedded therein in accordance with a predetermined embedding pattern, the cell aggregates each comprising a plurality of cells;

establishing a group of candidate cell types to be evaluated for use as a constituent of at least one of the plurality of cell aggregates based at least in part on the suitability of cells of the candidate cell types to perform a desired biological function;

executing a computer simulation based on parameters comprising interaction forces between two cells of the same type for each of the candidate cell types, an interaction force between one of the cells of each candidate cell type and the matrix, and an interaction force between two volume elements of the matrix to

predict structural evolutions of the pluralities of cells that are likely to occur for each cell type if said plurality of cell aggregates comprising cells of the respective candidate cell type are embedded in the matrix in accordance with the embedding pattern; and

selecting one of the candidate cell types that is predicted to result in the cell aggregates evolving into the desired three-dimensional structure for use in producing fused aggregates forming the desired structure.

24. The method of claim 23 further comprising:

forming said plurality of cell aggregates, at least one of the plurality of cell aggregates comprising a plurality of cells of the selected cell type;

embedding said plurality of cell aggregates in the matrix in accordance with the embedding pattern;

allowing at least one aggregate of said plurality of cell aggregates to fuse with at least one other aggregate of said plurality of cell aggregates to form the desired structure; and

separating the structure from the matrix.

25. The method of claim 23 wherein the step of executing a computer simulation comprises:

(i) creating a numerical model of a structure of said pluralities of cells of the plurality of cell aggregates and the matrix that is representative of an initial structure that said pluralities of cells and the matrix would have if said plurality of cell aggregates are embedded in the matrix in accordance with the embedding pattern;

(ii) estimating an initial total interaction energy of said pluralities of cells and the matrix if said plurality of cell aggregates comprise cells of a first candidate cell type and are embedded in the matrix in accordance with the embedding pattern;

(iii) simulating with a computer random movement of one of said pluralities of cells with respect to the remainder of said pluralities of cells and estimating a change in the total interaction energy caused by said movement;

(iv) updating the model to incorporate said movement if it lowers the total interaction energy; and

(v) repeating steps (iii) and (iv) until it is known whether the model evolves into a long-lived structure.

26. A computerized method of producing a plurality of fused aggregates forming a desired three-dimensional structure comprising:

identifying a matrix to be used to temporarily support a plurality of cell aggregates embedded therein in accordance with an embedding pattern, the cell aggregates each comprising a plurality of cells;

establishing a group of candidate embedding patterns to be evaluated for use as the embedding pattern by which said plurality of cell aggregates are to be embedded in the matrix based at least in part on similarity of the embedding pattern to the desired three-dimensional structure;

executing a computer simulation based on parameters comprising interaction forces between two of the cells, an interaction force between one of the cells and the

matrix, and an interaction force between two volume elements of the matrix to predict structural evolutions of the pluralities of cells that are likely to occur for each of the candidate embedding patterns if said plurality of cell are embedded in the matrix in accordance with the respective embedding pattern; and

selecting one of the candidate embedding patterns that is predicted to result in the cell aggregates evolving into the desired three-dimensional structure for use in producing fused aggregates forming the desired structure.

27. The method of claim 26 further comprising:
forming the matrix;

embedding said plurality of cell aggregates in the matrix in accordance with the selected embedding pattern;

allowing at least one aggregate of said plurality of cell aggregates to fuse with at least one other aggregate of said plurality of cell aggregates to form the desired structure; and

separating the structure from the matrix.

28. The method of claim 26 wherein the step of executing a computer simulation comprises:

(i) creating a numerical model of a structure of said pluralities of cells and the matrix that is representative of an initial structure that said pluralities of cells and the matrix would have if said plurality of cell aggregates are embedded in the matrix in accordance with a first candidate embedding pattern;

(ii) estimating an initial total interaction energy of said pluralities of cells and the matrix if said plurality of cell aggregates are embedded in the matrix in accordance with the first candidate embedding pattern;

(iii) simulating with a computer random movement of one of said pluralities of cells with respect to the remainder of said pluralities of cells and estimating a change in the total interaction energy caused by said movement;

(iv) updating the model to incorporate said movement if it lowers the total interaction energy; and

(v) repeating steps (iii) and (iv) until it is known whether the model evolves into a long-lived structure.

29. A composition comprising a plurality of cell aggregates, wherein each cell aggregate comprises a plurality of living cells, and wherein the cell aggregates are substantially uniform in size or shape.

30. The composition of claim 29 wherein the cell aggregates are selected from the group consisting of cylindrical cell aggregates, cuboidal cell aggregates, rod-like cell aggregates, and substantially spherical cell aggregates.

31. The composition of claim 30 wherein the cell aggregates are substantially spherical cell aggregates.

32. The composition of claim 31 wherein the cell aggregates are from about 100 microns to about 600 microns in diameter.

33. The composition of claim 29 wherein each cell aggregate comprises a plurality of living cells of a single cell type.

34. The composition of claim 33 wherein each cell aggregate further comprises extracellular matrix proteins.

35. The composition of claim 29 wherein each cell aggregate comprises a plurality of living cells of a first cell type and a plurality of living cells of at least one other cell type, the at least one other cell type being different from the first cell type.

36. The composition of claim 35 wherein each cell aggregate further comprises extracellular matrix proteins.

37. A printer cartridge comprising a storage vessel, wherein the composition of claim 29 is stored in the storage vessel.

38. A composition comprising a plurality of cell aggregates, wherein each cell aggregate comprises a plurality of living cells, and wherein the cell aggregates are substantially uniform in shape and size.

39. A method of preparing a plurality of cell aggregates, the method comprising:

preparing a cell suspension comprising a plurality of living cells;

centrifuging the cell suspension to form a cellular material comprising at least some of the plurality of living cells;

extruding the cellular material through an orifice; and

forming the extruded cellular material into cell aggregates of substantially uniform size or shape.

40. The method of claim 39 wherein the orifice is selected from the group consisting of a circular orifice and a square orifice.

41. The method of claim 40 wherein the orifice is a circular orifice having a diameter of from about 100 microns to about 600 microns.

42. The method of claim 39 wherein the cell aggregates are formed by slicing the extruded cellular material at regular intervals.

43. The method of claim 42 wherein the cell aggregates are cylindrical, and have a diameter equal to the height of the aggregates.

44. The method of claim 43 further comprising incubating the cell aggregates for about 1 to about 5 hours and agitating the cell aggregates.

45. An apparatus for producing a plurality of substantially uniform cell aggregates, the apparatus comprising:

an extrusion system adapted to receive a container holding a pellet comprising a plurality of living cells and to extrude the pellet through an orifice; and

a cutting system operable to cut the extrudate into a plurality of pieces as the extrudate is being extruded through the orifice.

46. The apparatus of claim 45 wherein the orifice is circular.

47. The apparatus of claim 45 further comprising a control unit operable to control operation of the extruding system and operation of the cutting system.

48. The apparatus of claim 47 wherein the control unit is operable to coordinate operation of the cutting system with operation of the extrusion system so the cutting system cuts the extrudate into substantially uniform pieces.

49. The apparatus of claim 47 wherein the cutting system comprises an actuator and a cutting blade, the actuator being operable to slide the cutting blade past the orifice.

50. The apparatus of claim 45 wherein the extruding system comprises a motor and a piston drivingly connected

to the motor, the motor being operable to advance the piston through a container containing said extrudate.

51. The apparatus of claim 50 in combination with said container.

52. A three-dimensional layered structure comprising:

at least one layer of a biocompatible matrix; and
a plurality of cell aggregates, each cell aggregate comprising a plurality of living cells;

wherein the cell aggregates are embedded in the at least one layer of biocompatible matrix in a predetermined pattern.

53. The structure of claim 52 wherein the cell aggregates are substantially uniform in size and shape.

54. The structure of claim 52 wherein the cell aggregates are cylindrical.

55. The structure of claim 54 wherein the cylindrical cell aggregates are from about 100 microns to about 600 microns in height.

56. The structure of claim 52 wherein the cell aggregates are substantially spherical.

57. The structure of claim 56 wherein the substantially spherical cell aggregates are between about 100 and about 600 microns in diameter.

58. The structure of claim 52 wherein each cell aggregate comprises a plurality of living cells of a single cell type.

59. The structure of claim 52 wherein each cell aggregate comprises a plurality of living cells of a first cell type and a plurality of living cells of a second cell type, the second cell type being different from the first cell type.

60. The structure of claim 52 wherein the plurality of cell aggregates comprises a plurality of cell aggregates of a first cell type and a plurality of cell aggregates of a second cell type, the second cell type being different from the first cell type.

61. The structure of claim 52 wherein the layer of biocompatible matrix is about 100 microns to about 600 microns thick.

62. The structure of claim 52 wherein the biocompatible matrix is selected from the group consisting of thermo-reversible gels, photo-sensitive gels, pH-sensitive gels, cell type specific gels, and combinations thereof.

63. The structure of claim 52 wherein the at least one layer of biocompatible matrix comprises at least two different types of biocompatible matrices.

64. The structure of claim 52 comprising:
a first layer of the biocompatible matrix; and
a second layer of the biocompatible matrix deposited
on the first layer;

wherein the cell aggregates are embedded in the
first layer and in the second layer in a predetermined
pattern.

65. The structure of claim 64 further comprising at
least one additional layer of the biocompatible matrix
deposited on the second layer, wherein the cell
aggregates are embedded in the first layer, the second
layer, and the at least one additional layer in a
predetermined pattern.

66. The structure of claim 64 wherein the first
layer comprises a type of biocompatible matrix that is
different from the type of biocompatible matrix in the
second layer.